Supporting Information

Zhang et al. 10.1073/pnas.0903030106

SI Methods

Culture Media and Growth Conditions. *Cupriavidus oxalaticus* str. OX1 (DSM 1105^T) and *Cupriavidus necator* str. H16 (previously known as *Ralstonia eutropha*, DSM 428) were cultivated in minimal medium described by Dijkhuizen and Harder (1). We used 15 mM rather than 20 mM phosphate buffering (pH 7.2) for pH control. *Escherichia coli* K-12 str. MG1655 was cultivated in M9 minimal media (2). Glucose (22.2 mM) was replaced with other carbon sources at 15 mM for the appropriate experiments (see Table 1). All minimal media cultures were amended with EDTA-chelated trace elements formulated according to Flagan et al. (3). *Rhodopseudomonas palustris* str. TIE-1 was cultivated in freshwater minimal medium (4) according to Rashby et al. (5) containing 20 mM bicarbonate buffer and 20 mM thiosulfate for photoautotrophic growth, 20 mM N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer and 20 mM acetate for photoheterotrophic growth, and 20 mM acetate for aerobic heterotrophic growth. Growth substrates were potassium oxalate monohydrate (Sigma), sodium formate (Mallinckrodt), anhydrous D-glucose (Mallinckrodt), anhydrous sodium acetate (Sigma), sodium succinate hexahydrate (≥ 99%, Sigma), sodium pyruvate (≥ 99%, Sigma), D-(−)-fructose (≥ 99%, Sigma), and sodium D-gluconate (97%, Sigma). Aerobic cultures (0.5 L) of *C. oxalaticus*, *C. necator*, and *E. coli* were grown shaking at 200 or 250 rpm in 1-L combusted Pyrex flasks. *Cupriavidus* was cultivated at 30 °C, *E. coli* at 37 °C. Phototrophic cultures of *R. palustris* were incubated in 2-L flasks with 1 L of N_2 headspace and 2,000-lux illumination at room temperature. Aerobic heterotrophic cultures (1 L) were grown at 30 °C, shaking at 250 rpm in the dark.

Isotopic Analyses. The δD values of the most abundant FAMEs were measured on a ThermoScientific GC coupled to a Delta$^+$XP isotope-ratio mass spectrometer (IRMS) via the GC/TC pyrolysis interface. Chromatographic conditions were identical as for GC/MS analysis, and peaks were identified by retention order and relative height. The H_3-factor was calibrated daily using multiple peaks of H_2 reference gas at varying intensity, and was stable at ≈4.7–4.8 ppm/mV. Data are reported in the conventional δD notation versus the VSMOW standard, and are corrected for the addition of methyl H in the derivative. The δD value of added methyl H used for this correction was determined by analyzing the dimethyl derivative of phthalic acid for which the δD value of ring H is known. Replicate analyses were performed for all samples except Co1-Co4, and an external standard containing either 16 *n*-alkanes (*R. palustris* and *E. coli* data) or 8 FAMEs (*Cupriavidus* data) of known δD value was analyzed every 5th injection. The root-mean-square (RMS) error of all external standards analyzed with these samples was 2.9‰. Typical precision (1σ) based on multiple analyses of analytes was 3.4‰. The δD of culture media was measured on a Los Gatos Research DLT-100 liquid water isotope analyzer. This instrument measures by absorption spectroscopy, and has been evaluated in detail by Lis et al. (6). Six sequential aliquots (0.8 μL of each) of each sample were injected, with the first 3 discarded, to minimize memory effects.

Substrate δD values were measured by equilibrating selected aliquots with at least 2 waters of differing D/H ratio (as steam) to control for the presence of exchangeable H before conversion to H_2 by sequential combustion/reduction (7) and analysis by dual-inlet IRMS. The isotope ratio of nonexchangeable H was then calculated by mass balance from the 2 exchanged samples (8). Because of significant uncertainties associated with this correction, uncertainties in reported values may be as high as ± 20‰. Gluconate was also analyzed by this method, but did not yield reliable results for unknown reasons. The extreme D enrichment of formate indicated by this method is similar to that obtained for a separate formate sample, from a different supplier, measured by a different lab using a different analytical method (9) and is considered reliable.

1. Dijkhuizen L, Harder W (1975) Substrate inhibition in Pseudomonas oxalaticus OX1: A kinetic study of growth inhibition by oxalate and formate using extended cultures. *Antonie van Leeuwenhoek* 41:135–146.
2. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Plainview, NY).
3. Flagan S, Ching WK, Leadbetter JR (2003) Arthrobacter strain VAI-A utilizes acyl-homoserine lactone inactivation products and stimulates quorum signal biodegradation by Variovorax paradoxus. *Appl Environ Microbiol* 69:909–916.
4. Ehrenreich A, Widdel F (1994) Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Appl Environ Microbiol* 60:4517–4526.
5. Rashby SE, Sessions AL, Summons RE, Newman DK (2007) Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proc Natl Acad Sci USA* 104:15099.
6. Lis G, Wassenaar LI, Hendry MJ (2008) High-precision laser spectroscopy D/H and $^{18}O/^{16}O$ measurements of microliter natural water samples. *Anal Chem* 80:287–293.
7. Schimmelmann A, DeNiro M (1993) Preparation of organic and water hydrogen for stable isotope analysis: Effects due to reaction vessels and zinc reagent. *Anal Chem* 65:789–792.
8. Schimmelmann A (1991) Determination of the concentration and stable isotopic composition of nonexchangeable hydrogen in organic matter. *Anal Chem* 63:2456–2459.
9. Campbell BJ, Li C, Sessions AL, Valentine DL (2009) Hydrogen isotopic fractionation in lipid biosynthesis by H_2-consuming Desulfobacterium autotrophicum. *Geochim Cosmochim Acta* 73:2744–2757.

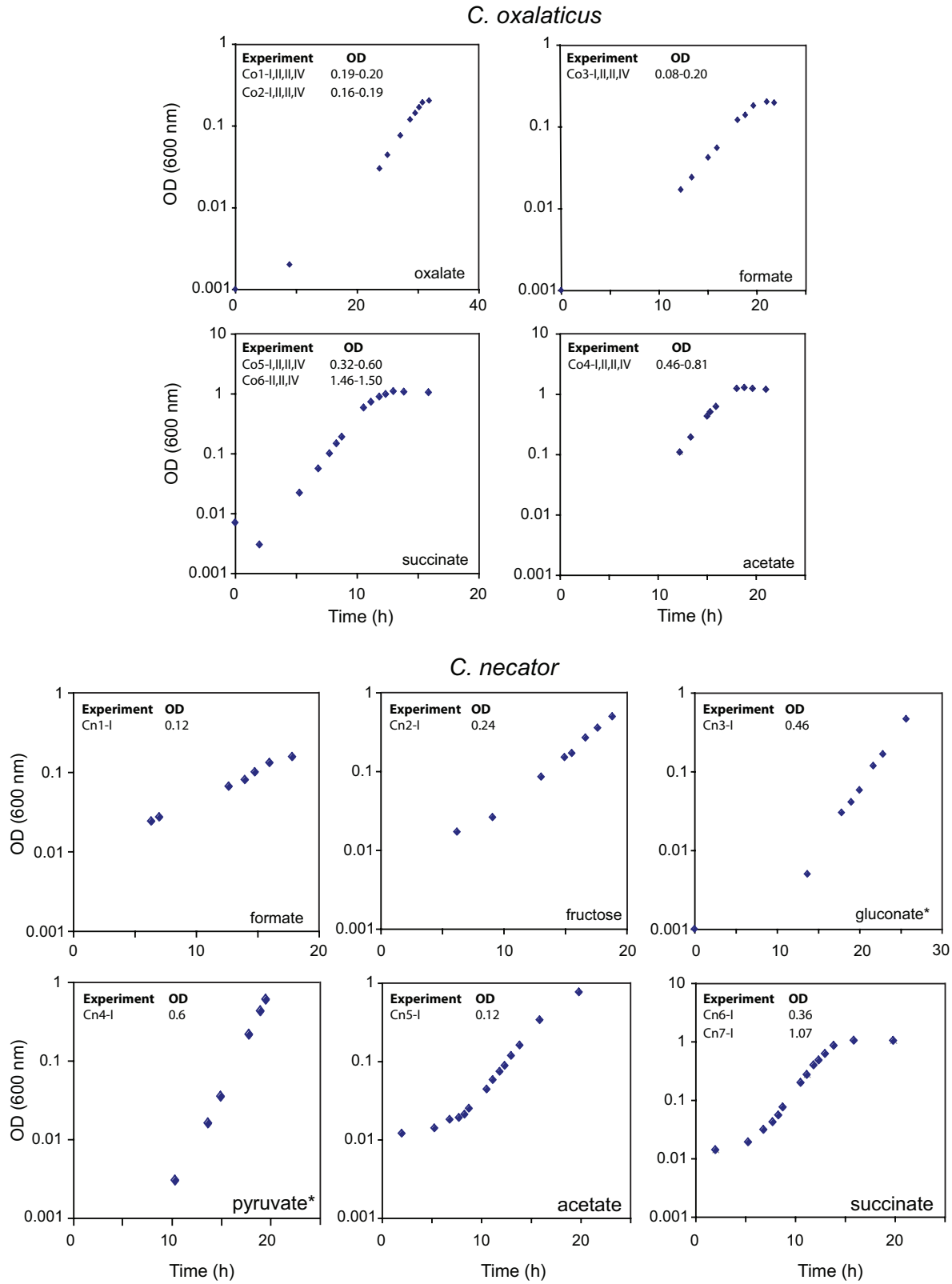

Fig. S1. Representative growth curves for *C. oxalaticus*, *C. necator*, *E. coli*, and *R. palustris* cultures on selected substrates. OD_{600nm} values at harvest are listed. For most substrates, plotted growth data are from a single culture grown to stationary phase to define the growth curve, but not then analyzed. Panels containing substrates marked with asterisks show the growth curve from a culture that was harvested for isotopic analysis, generally in mid-log phase.

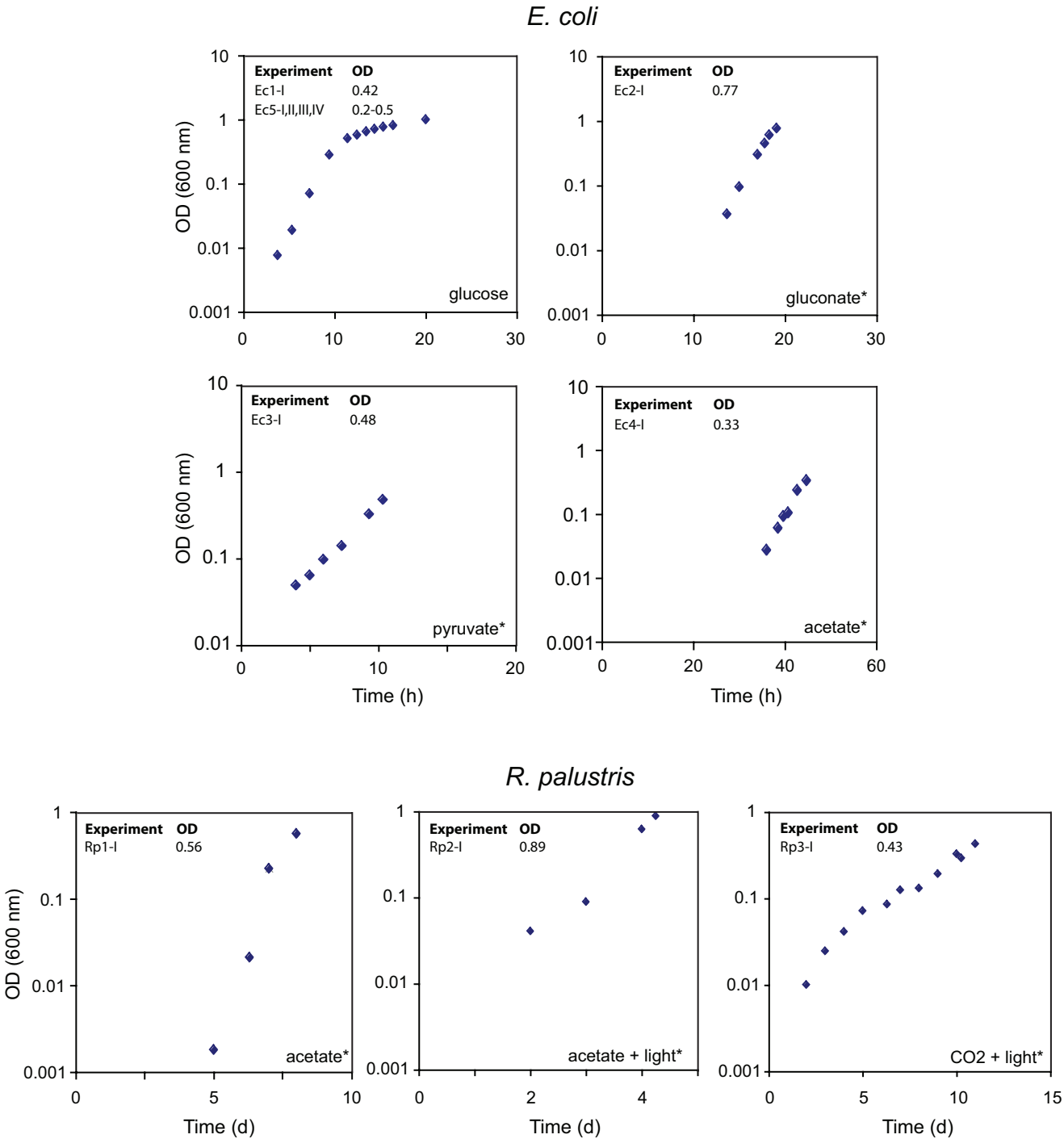

Fig. S1. continued.

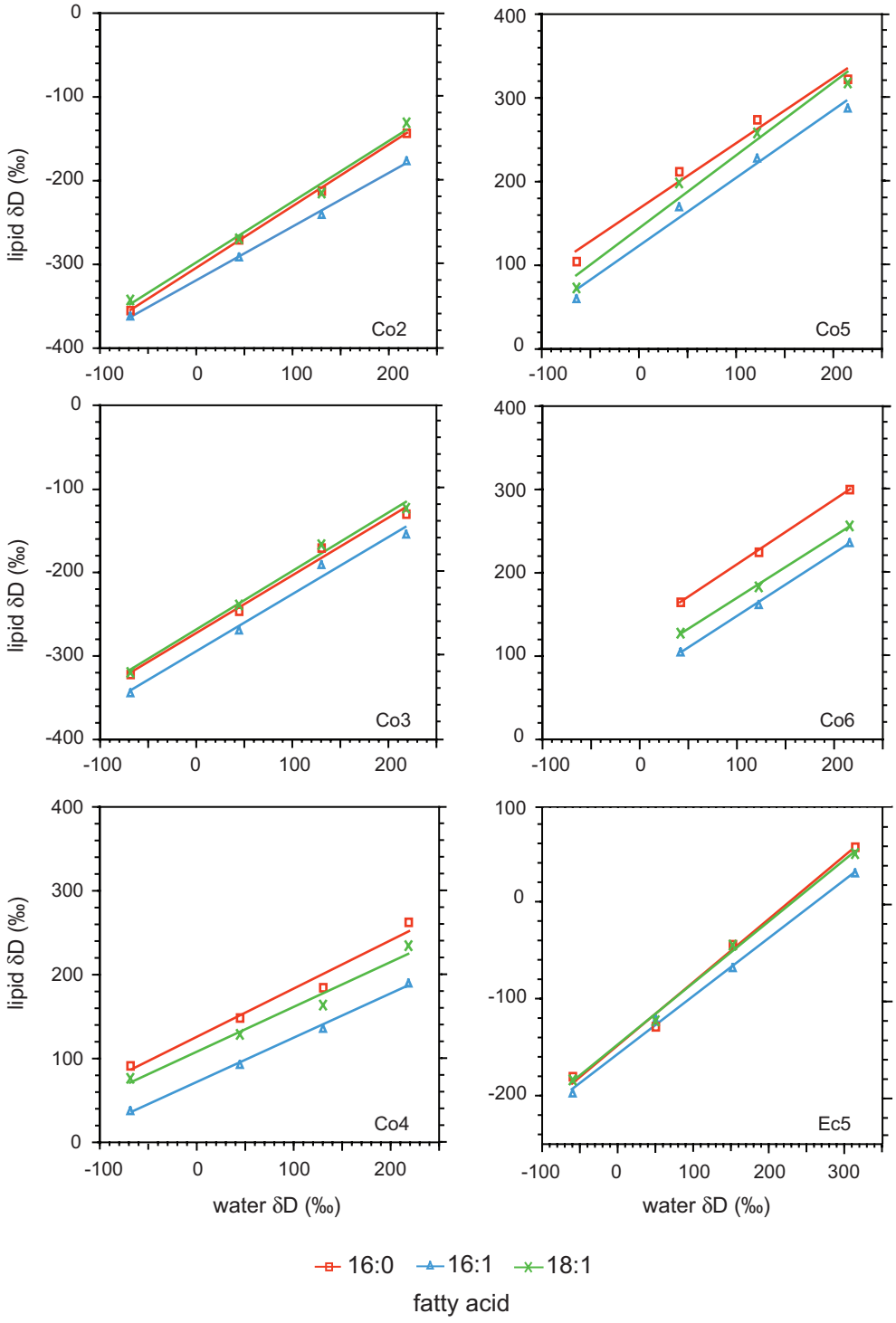

Fig. S2. Relationship between fatty acid and water δD values for *C. oxalaticus* and *E. coli* cultures. The slope of each regression curve is equivalent to $X_w\alpha_{l/w}$. Culture numbers are labeled in the lower right of each plot.

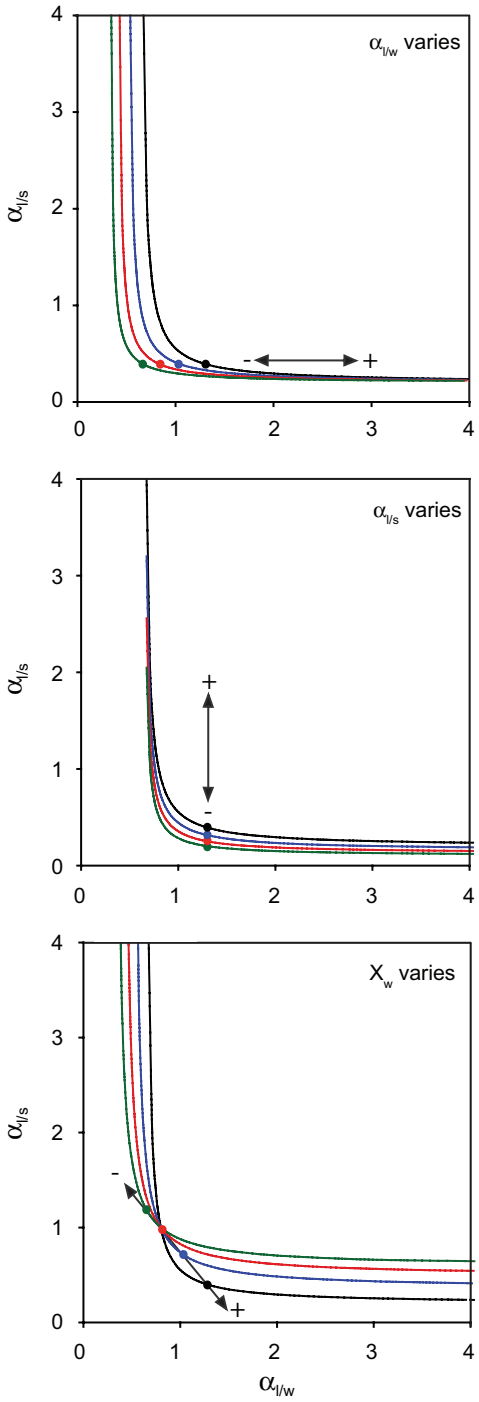

Fig. S3. Fractionation curves for hypothetical sets of cultures that differ only in a single parameter ($\alpha_{l/s}$, $\alpha_{l/w}$, or X_w). The plotted curves reflect 20% incremental changes in the specified parameter and arrows indicate the direction of change for an entire curve. The effects can be treated as independent, such that the result of changing 2 parameters can be estimated by vector addition. Filled circles mark $X_w = 0.5$. To account for curves that shift up and to the right (e.g., those in Fig. 3), both $\alpha_{l/s}$ and $\alpha_{l/s}$ must simultaneously change.

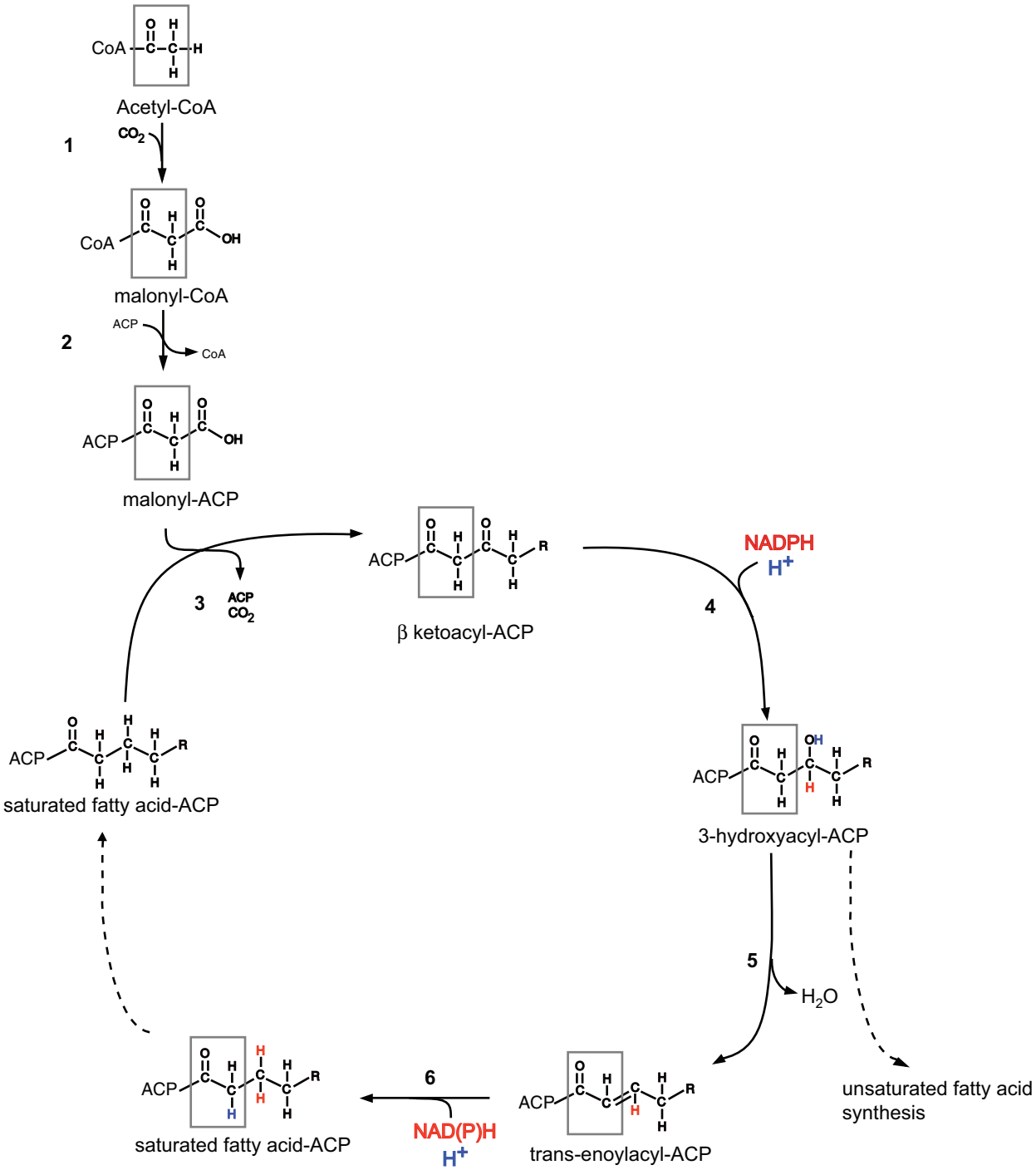

Fig. S4. Fatty acid biosynthetic pathway highlighting cellular sources of H (refs. 1–5). (1) Acetyl-CoA carboxylase, which is regulated to control carbon flux into lipids. (2) Malonyl CoA-ACP transacylase. (3) β-ketoacyl ACP synthase (FabB, FabF, FabH). FabH controls biosynthesis initiation and fatty acid composition based on acyl-CoA specificity, whereas FabB and FabF catalyze subsequent rounds of elongation by condensing malonyl-ACP with acyl-ACP. (4) β-ketoacyl ACP reductase (FabG). (5) β-hydroxyacyl ACP dehydratase (FabZ, FabA). (6) Enoyl ACP reductase (FabI, FabK, FabL).

1. Marrakchi H, Zhang Y, Rock CO (2002) Mechanistic diversity and regulation of Type II fatty acid synthesis. *Biochem Soc Trans* 30:1050–1055.
2. Magnuson K, Jackowski S, Rock CO, Cronan JE (1993) Regulation of fatty acid biosynthesis in Escherichia coli. *Microbiol Molec Biol Rev* 57:522–542.
3. Campbell JW, Cronan JE (2001) Bacterial fatty acid biosynthesis: Targets for antibacterial drug discovery. *Annu Rev Microbiol* 55:305–332.
4. Rock CO, Cronan JE (1996) Escherichia coli as a model for the regulation of dissociable (type II) fatty acid biosynthesis. *Biochim Biophys Acta* 1302:1–16.
5. White S, Zheng J, Zhang Y, Rock CO (2005) The structural biology of Type II fatty acid biosynthesis. *Annu Rev Biochem* 74:791–831.

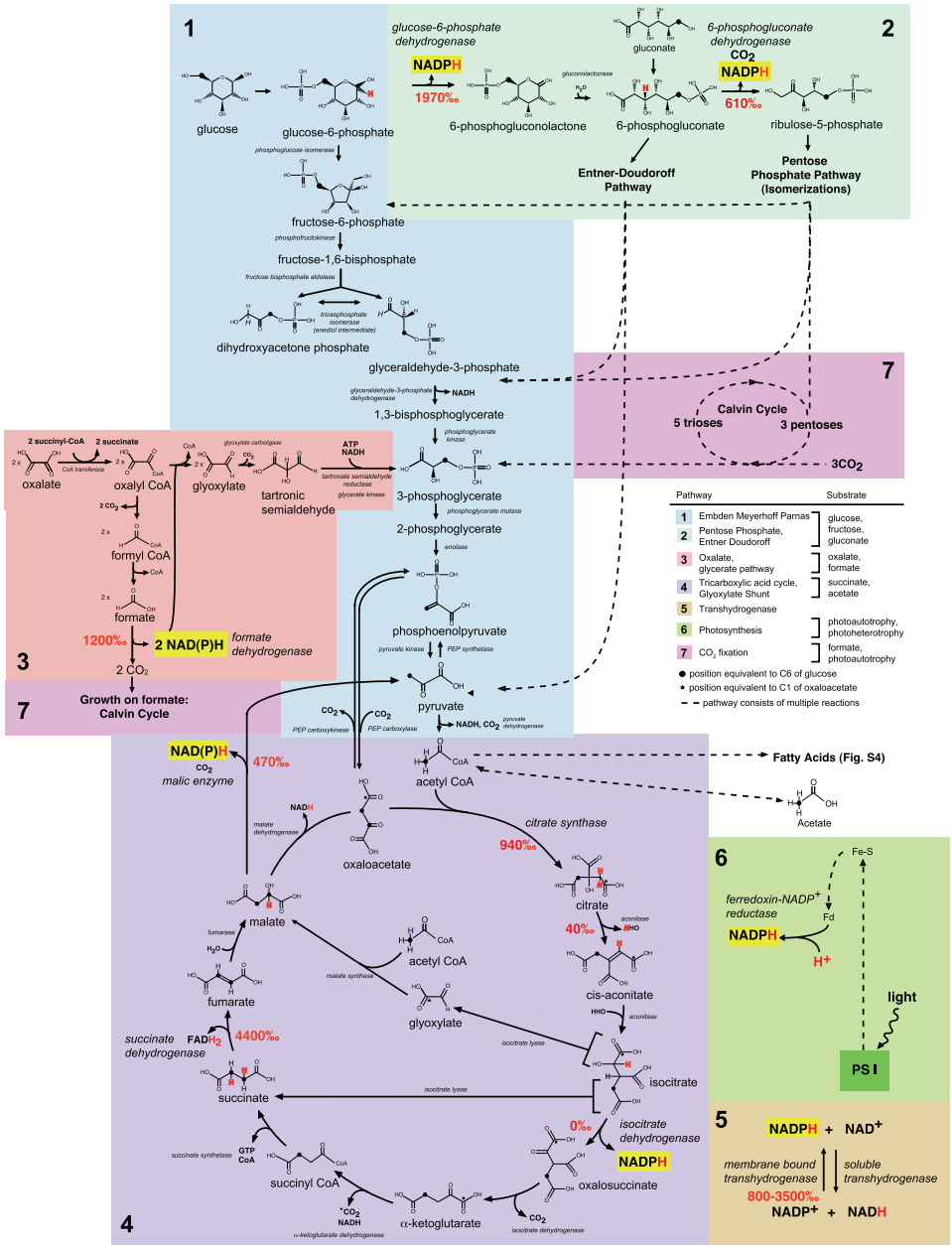

Fig. S5. Schematic summary of major central metabolic pathways highlighting the most important sources of NADPH reduction (refs. 1–5). (1) Embden–Meyerhoff–Parnas Pathway (glycolysis). (2) Oxidative pentose phosphate pathway and Entner–Doudoroff pathway (sugar degradation, glycolysis). (3) Oxalate degradation and assimilation via the glycerate pathway. (4) Tricarboxylic acid cycle and glyoxylate shunt. (5) Transhydrogenase. (6) Light reactions of photosynthesis. (7) Calvin cycle for CO_2 fixation. Reactions producing NADPH or potentially having strong influence on the isotopic composition of NADPH are highlighted. D/H fractionations (in permil notation), corresponding to isotope effects $^{H}k/^{D}k$ or $^{D}(V/K)$, are indicated by red numbers (6–10). Filled circles denote positions equivalent to C-6 of glucose and the methyl group of acetate. Filled stars denote the position equivalent to C-1 of oxaloacetate.

1. Gottschalk G (1986) *Bacterial Metabolism* (Springer-Verlag, New York).
2. Ingraham JL, Maaløe O, Neidhardt FC (1983) *Growth of the Bacterial Cell* (Sinauer, Sunderland, MA).
3. White D (2000) *The Physiology and Biochemistry of Prokaryotes* (Oxford Univ Press, New York).
4. Quayle JR (1961) Metabolism of C1 compounds in autotrophic and heterotrophic microorganisms. *Annu Rev Microbiol* 15:119–152.
5. Kornberg HL, Elsden SR (1961) The metabolism of 2-carbon compounds by microorganisms. *Adv Enzymol Relat Subj Biochem* 23:401–470.
6. Retey J, et al. (1970) Stereochemical studies of the exchange and abstraction of succinate hydrogen on succinate dehydrogenase. *Eur J Biochem* 14:232–242.
7. Thomson JF, Nance SL, Bush KJ, Szczepanik PA (1966) Isotope and solvent effects of deuterium on aconitase. *Arch Biochem Biophys* 117:65–74.
8. O'Leary MH (1989) Multiple isotope effects on enzyme-catalyzed reactions. *Annu Rev Biochem* 59:377–401.
9. Jackson JB, Peake SJ, White SA (1999) Structure and mechanism of proton-translocating transhydrogenase. *FEBS Lett* 464:1–8.
10. Lenz H, et al. (1971) Stereochemistry of *si*-citrate synthase and ATP-citrate-lyase reactions. *Eur J Biochem* 24:207–215.

Table S1. Relative abundances of fatty acids in bacterial cultures

	Fatty acid*											
Culture	12:0	14:1	14:0	15:0	16:1	16:0	cyc17	17:0	18:1	18:0	cyc19	19:0
Co1-I	—	—	—	—	0.39	0.38	—	—	0.22	—	—	—
Co1-II	—	—	—	—	0.35	0.39	—	—	0.25	0.01	—	—
Co1-III	—	—	—	—	0.36	0.36	0.01	—	0.27	0.01	—	—
Co1-IV	—	—	—	—	0.37	0.35	0.01	—	0.27	0.01	—	—
Co2-I	—	—	—	—	0.38	0.34	—	—	0.26	0.01	—	—
Co2-II	—	—	0.02	—	0.40	0.30	0.01	—	0.26	0.01	—	—
Co2-III	—	—	—	—	0.39	0.31	—	—	0.29	—	—	—
Co2-IV	—	—	—	—	0.37	0.33	—	—	0.29	0.01	—	—
Co3-I	—	—	—	—	0.40	0.34	—	—	0.25	0.01	—	—
Co3-II	—	—	—	—	0.40	0.34	—	—	0.25	0.01	—	—
Co3-III	—	—	—	—	0.39	0.36	—	—	0.24	0.01	—	—
Co3-IV	—	—	—	—	0.40	0.35	—	—	0.24	0.01	—	—
Co4-I	—	—	—	—	0.39	0.38	—	—	0.22	0.01	—	—
Co4-II	—	—	—	—	0.39	0.41	—	—	0.19	—	—	—
Co4-III	—	—	—	—	0.40	0.41	—	—	0.17	0.01	—	—
Co4-IV	—	—	0.01	—	0.39	0.42	—	—	0.17	0.01	—	—
Co5-I	—	—	0.03	—	0.39	0.33	—	—	0.25	0.01	—	—
Co5-II	—	—	—	—	0.45	0.38	—	—	0.16	—	—	—
Co5-III	—	—	—	—	0.43	0.40	—	—	0.17	—	—	—
Co5-IV	—	—	—	—	0.41	0.40	—	—	0.18	—	—	—
Co6-II	—	—	—	—	0.36	0.38	0.05	—	0.20	—	—	—
Co6-III	—	—	—	—	0.35	0.35	0.08	—	0.21	—	—	—
Co6-IV	—	—	—	—	0.41	0.36	0.06	—	0.17	—	—	—
Cn1-I	—	—	0.01	—	0.39	0.32	0.02	—	0.26	0.01	—	—
Cn2-I	—	—	0.02	—	0.42	0.29	0.01	—	0.25	0.01	—	—
Cn3-I	—	—	—	—	0.36	0.33	0.01	—	0.30	—	—	—
Cn4-I	—	—	0.01	—	0.43	0.39	—	—	0.18	—	—	—
Cn5-I	—	—	0.02	—	0.42	0.32	—	—	0.24	—	—	—
Cn6-I	—	—	0.02	—	0.39	0.34	—	—	0.23	0.01	—	—
Cn7-I	—	—	0.05	—	0.10	0.36	0.32	—	0.14	—	0.03	—
Ec1-I	0.01	—	0.04	0.01	0.21	0.45	0.13	0.01	0.14	—	0.01	—
Ec2-I	0.01	—	0.03	—	0.19	0.49	0.15	0.01	0.12	—	—	—
Ec3-I	—	—	0.02	—	0.16	0.50	0.18	—	0.11	0.02	0.01	—
Ec4-I	—	—	0.04	—	0.12	0.53	0.21	—	0.09	—	0.02	—
Ec5-I	0.02	—	0.04	0.01	0.20	0.43	0.11	—	0.17	—	0.01	—
Ec5-II	0.02	—	0.04	—	0.02	0.46	0.32	0.01	0.04	—	0.08	—
Ec5-III	0.02	—	0.04	0.01	0.15	0.44	0.16	0.01	0.15	—	0.01	—
Ec5-IV	0.02	—	0.04	0.01	0.01	0.50	0.22	0.01	0.15	—	0.02	—
Ec6-I	0.03	0.01	0.06	0.02	0.26	0.38	0.07	0.01	0.16	0.01	—	—
Ec6-II	0.03	0.01	0.07	0.02	0.29	0.38	0.04	0.01	0.15	—	—	—
Ec6-III	0.02	0.01	0.05	0.02	0.33	0.42	0.14	—	0.01	—	—	—
Rp1-I	—	—	—	—	0.01	0.08	—	—	0.53	0.21	0.15	0.02
Rp2-I	—	—	—	—	0.03	0.14	—	—	0.65	0.11	0.07	—
Rp3-I	—	—	—	—	0.03	0.15	—	—	0.69	0.10	0.01	—

*12:0, lauric acid; 14:1, myristoleic acid; 14:0, myristic acid; 15:0, pentadecanoic acid; 16:1, palmitoleic acid; 16:0, palmitic acid; cyc17, cyclopropyl-heptadecanoic acid; 17:0, heptadecanoic acid; 18:1, oleic acid; 18:0, stearic acid; cyc19, cyclopropyl-nonadecanoic acid; 19:0, nonadecanoic acid. Relative abundances are calculated from TIC peak areas of FAMEs as the fraction of total quantified fatty acids.

Table S2. Measured δD values of fatty acids and culture media water

		Fatty Acid*									
Culture	$n^{\dagger}$	16:1	σ	16:0	σ	cyc17	σ	18:1	σ	18:0	σ	Medium, δD_w
Co1-I	1	−356	—	−343	—	—	—	−338	—	—	—	−68.6
Co1-II	1	−281	—	−266	—	—	—	−260	—	—	—	44.6
Co1-III	1	−219	—	−198	—	—	—	−193	—	—	—	130.3
Co1-IV	1	−174	—	−148	—	—	—	−140	—	—	—	218.3
Co2-I	1	−362	—	−355	—	—	—	−342	—	—	—	−68.6
Co2-II	1	−291	—	−270	—	—	—	−269	—	—	—	44.6
Co2-III	1	−240	—	−212	—	—	—	−215	—	—	—	130.3
Co2-IV	1	−176	—	−143	—	—	—	−131	—	—	—	218.3
Co3-I	1	−344	—	−322	—	—	—	−319	—	—	—	−68.6
Co3-II	1	−269	—	−246	—	—	—	−239	—	—	—	44.6
Co3-III	1	−191	—	−170	—	—	—	−167	—	—	—	130.3
Co3-IV	1	−154	—	−130	—	—	—	−123	—	—	—	218.3
Co4-I	1	38	—	92	—	—	—	76	—	—	—	−68.6
Co4-II	1	93	—	149	—	—	—	129	—	—	—	44.6
Co4-III	1	129	—	187	—	—	—	167	—	—	—	130.3
Co4-IV	1	190	—	263	—	—	—	234	—	—	—	218.3
Co5-I	2	64	1.7	109	3.0	—	—	77	0.8	—	—	−64.3
Co5-II	3	176	1.0	219	1.4	—	—	205	1.6	—	—	41.1
Co5-III	3	235	2.5	282	1.2	—	—	266	2.8	—	—	121.1
Co5-IV	3	296	3.6	331	4.2	—	—	326	5.0	—	—	214.1
Co6-II	3	106	1.3	166	1.1	—	—	128	4.1	—	—	41.1
Co6-III	3	163	0.7	226	1.4	—	—	184	0.7	—	—	121.1
Co6-IV	3	238	1.6	302	3.2	—	—	258	1.8	—	—	214.1
Cn1-I	4	−298	3.5	−294	3.5	—	—	−287	8.2	—	—	−68.3
Cn2-I	2	−137	0.1	−101	1.4	—	—	−110	2.1	—	—	−65.5
Cn3-I	2	−124	1.9	−124	3.4	—	—	−109	4.1	—	—	−68.1
Cn4-I	4	−12	2.2	26	3.0	—	—	8	5.8	—	—	−64.4
Cn5-I	4	71	2.1	127	11.8	—	—	101	1.8	—	—	−68.5
Cn6-I	2	51	1.7	89	1.5	—	—	62	0.7	—	—	−68.6
Cn7-I	2	−35	2.6	−3	0.5	−11	1.9	−34	2.2	—	—	−68.6
Ec1-I	2	−176	2.7	−178	0.3	−160	2.8	−173	2.2	—	—	−61.9
Ec2-I	2	−196	4.3	−190	3.3	−166	1.4	−187	4.6	—	—	−62.2
Ec3-I	4	−124	3.8	−120	5.8	−112	3.7	−108	5.2	—	—	−68.1
Ec4-I	2	−23	2.2	−12	3.1	−7	0.7	−5	0.5	—	—	−62.4
Ec5-I	2	−197	0.0	−180	3.3	−178	2.7	−183	2.7	—	—	−60.0
Ec5-II	2	−122	10.4	−128	1.1	−121	1.3	−122	0.6	—	—	49.9
Ec5-III	2	−68	1.8	−44	1.4	−52	0.7	−44	0.5	—	—	152.0
Ec5-IV	2	30	0.4	57	0.2	41	1.2	50	3.2	—	—	314.0
Ec6-I	2	−152	0.6	−143	0.0	−139	1.4	−121	0.4	—	—	−60.0
Ec6-II	2	−98	0.3	−83	1.6	−116	2.9	−61	0.7	—	—	49.9
Ec6-III	2	−58	3.4	−34	0.5	−70	4.9	−20	7.1	—	—	152.0
Rp1-I	2	—	—	−87	1.4	—	—	−77	4.0	−37	0.5	−53.6
Rp2-I	2	−169	3.6	−185	1.4	—	—	−173	2.1	−157	1.4	−53.6
Rp3-I	2	—	—	−220	0.9	—	—	−229	1.2	−208	1.2	−53.6

*Fatty acid structures for corresponding abbreviations are listed in Table S1. Tabulated values are the average δD values for replicate analyses, in permil. Values of σ are calculated from replicate analyses.

†Number of replicate measurements for fatty acids

Table S3. Coefficients for regression of R_I on R_W and their standard errors (SE). Intercepts and their standard errors are x10^6

	16:1					16:0					18:1				
Cultures	Slope	SE	Intercept	SE	R^2	Slope	SE	Intercept	SE	R^2	Slope	SE	Intercept	SE	R^2
Co1-I,II,II,IV	0.64	0.03	7.22	4.77	1.00	0.69	0.03	2.83	4.77	1.00	0.70	0.02	1.72	3.48	1.00
Co2-I,II,II,IV	0.64	0.02	6.25	3.21	1.00	0.73	0.01	−5.70	2.13	1.00	0.72	0.05	−3.60	8.88	0.99
Co3-I,II,II,IV	0.69	0.06	3.04	10.25	0.98	0.69	0.05	5.97	8.62	0.99	0.70	0.04	4.92	7.50	0.99
Co4-I,II,II,IV	0.52	0.04	85.80	6.30	0.99	0.58	0.06	85.07	10.57	0.98	0.54	0.05	88.72	8.85	0.98
Co5-I,II,II,IV	0.83	0.07	46.55	12.18	0.99	0.80	0.09	58.07	14.58	0.98	0.89	0.10	40.34	16.90	0.98
Co6-II,II,IV	0.76	0.03	47.99	4.45	1.00	0.79	0.02	53.88	2.91	1.00	0.75	0.03	53.98	4.62	1.00
Ec5-I,II,III,IV	0.60	0.02	37.83	3.12	0.99	0.65	0.04	31.70	7.03	0.99	0.63	0.03	34.08	4.38	0.99
Ec6-I,II,III	0.44	0.03	67.19	5.28	0.99	0.52	0.02	57.99	3.42	0.99	0.48	0.04	67.41	6.42	0.99